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Michael S. Gaffrey

*Washington University School of Medicine in St. Louis*

Deanna M. Barch

*Washington University School of Medicine in St. Louis*

Joan L. Luby

*Washington University School of Medicine in St. Louis*

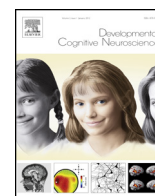
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# Amygdala reactivity to sad faces in preschool children: An early neural marker of persistent negative affect

Michael S. Gaffrey<sup>a,\*</sup>, Deanna M. Barch<sup>a,b,c</sup>, Joan L. Luby<sup>a</sup>

<sup>a</sup> Washington University in St. Louis, Department of Psychiatry, Campus Box: 8511, 660 South Euclid Avenue, Saint Louis, MO 63110, United States

<sup>b</sup> Washington University in St. Louis, Department of Psychology, Campus Box: 8511, 660 South Euclid Avenue, Saint Louis, MO 63110, United States

<sup>c</sup> Washington University in St. Louis, Department of Radiology, Campus Box: 8511, 660 South Euclid Avenue, Saint Louis, MO 63110, United States



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## ABSTRACT

**Background:** Elevated negative affect is a highly salient risk factor for later internalizing disorders. Very little is known about the early neurobiological correlates of negative affect and whether they associate with developmental changes in negative emotion. Such information may prove critical for identifying children deviating from normative developmental trajectories of negative affect and at increased risk for later internalizing disorders. The current study examined the relationship between amygdala activity and negative affect measured concurrently and approximately 12 months later in preschool-age children.

**Method:** Amygdala activity was assessed using functional magnetic resonance imaging in 31 medication-naïve preschool age children. Negative affect was measured using parent report both at the time of scan and 12 months later.

**Results:** Negative affect at baseline was positively correlated with right amygdala activity to sad faces, right amygdala activity to happy faces, and left amygdala activity to happy faces. Right amygdala activity to sad faces also positively predicted parent-reported negative affect 12 months later even when negative affect reported at baseline was controlled.

**Conclusions:** The current findings provide preliminary evidence for amygdala activity as a potential biomarker of persistent negative affect during early childhood and suggest future work examining the origins and long-term implications of this relationship is necessary.

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## 1. Introduction

Disrupted processing of negative affect is one of the most clinically salient characteristics of risk for later internalizing disorders, including depression and anxiety (Wilson et al., 2014; Karevold et al., 2009). Research continues to suggest that understanding early brain behavior relationships may be critical for the identification and prevention of later occurring psychopathology. However, very little is known about the neurobiological correlates of negative affect processing in very young children and whether such correlates can inform individual differences in negative affective reactivity at later time points. A large body of research consistently suggests that the amygdala plays an important role in the processing of emotionally salient information (Pessoa, 2010), and associations between heightened amygdala reactivity and elevated negative affect have been established in multiple study samples (Barch et al., 2012; Peluso et al., 2009; Henderson et al., 2014;

Swartz et al., 2015). Nevertheless, very little is known about this relationship in early childhood and whether amygdala reactivity at this age is predictive of future negative affective behavior. Given the challenges associated with reliably measuring behavioral markers of negative affect at this age using common approaches (e.g., potential for biased parent report, failure to successfully elicit negative responses to lab-based challenges, etc.), the exploration of neural markers of negative affect may hold promise for identifying highly objective and potentially more robust early markers of risk. In addition, understanding the developmental neurobiology of negative affect is likely to clarify our understanding of elevated negative affect as a feature common to many psychiatric conditions and to further inform mechanisms of change in transdiagnostic treatments targeting early development (Insel, 2014).

Research examining the relationship between early negative affect and later internalizing psychopathology, such as anxiety and depression, has suggested that elevated negative affect during early childhood is an important marker of risk. For example, at the symptom level, infants rated as 'fussy' and 'difficult to soothe' by their parents are also more likely to have higher scores on maternal ratings of depression and anxiety at 5 years of age (Cote et al., 2009).

\* Corresponding author.

E-mail address: [gaffrey@wustl.edu](mailto:gaffrey@wustl.edu) (M.S. Gaffrey).

Similarly, high negative emotionality scores during the infancy and preschool periods have been associated with elevated parental ratings of anxiety and depression symptoms during later school age and adolescence (Karevold et al., 2009; Dougherty et al., 2011). More recent data suggest a similar phenomenon when diagnostic outcomes are considered. Specifically, elevations in negative affect at 6 years of age have been found to significantly increase the odds of receiving a diagnosis of depression at 18 years old (Bould et al., 2014). Studies investigating early irritability (i.e., easy annoyance and touchiness characterized by anger and temper outbursts) have also suggested a strong link between elevations in negative affect and later psychopathology. Of particular relevance for identifying young children at increased risk for psychopathology, a recent study reported that irritability measured at 3 years of age was predictive of depression and oppositional defiant disorder (ODD) diagnoses 3 years later (i.e., 6 years of age) over and above baseline diagnostic status (Dougherty et al., 2013). Further, using measures controlling for item overlap, the authors also found that irritability measured when children were 3 years old predicted increases in dimensional measures of depression, ODD, and functional impairment at 6 years of age.

Amygdala reactivity to emotional stimuli in healthy young children has been demonstrated (Gee et al., 2013; Todd et al., 2011; Perlman and Peltz, 2011), but to date only one has examined its relationship with early childhood psychopathology. A study of face processing in preschool age children with and without a very early occurring form of depression found increased activity in the right amygdala of depressed preschoolers (Gaffrey et al., 2013). Individual differences in negative affect and amygdala activity at this age were also explored. Heightened right amygdala activity while viewing facial expressions of emotion was found to be correlated with elevated parental reports of negative affect in both depressed and healthy preschoolers, matching the few dimensional studies of amygdala activity and depression severity in children and adolescents (Henderson et al., 2014; Barch et al., 2012). As such, this study suggested an important link between concurrent negative affect and amygdala function in preschool age children. However, it left unanswered whether amygdala activity during the preschool period is predictive of future negative affect, a key question for understanding whether trajectories of early emotional development and amygdala function are related.

The goal of the current study was to examine the relationship between amygdala activity during face viewing and negative affect at the time of scan (i.e., baseline) and approximately 12 months later in preschool age children. Based on our previous neuroimaging work including healthy and depressed preschoolers (Gaffrey et al., 2013), we expected that elevated amygdala activity while viewing facial expressions of emotion would be associated with increased parent reported negative affect at baseline. Further, given longitudinal studies suggesting the relative stability of negative affect across development (Neppel et al., 2010), we anticipated that such amygdala activity would predict unique variance in negative affect at follow-up. Given that fMRI research examining the relationship between negative affect and specific facial expressions of emotion in children has been limited, a strong a priori hypothesis as to which face type(s) would be related to parent reported negative affect was not made.

## 2. Methods

### 2.1. Study participants

Four-to-six year old children were recruited from pediatrician's offices, daycare facilities, and other community resources throughout the greater St. Louis metropolitan area using a

screening checklist (Preschool Feelings Checklist (Luby et al., 2004) [PFC]) to identify preschoolers with elevated depressive symptoms and a healthy control group. Caregivers indicating that their preschoolers were at "low" ( $\leq 1$  PFC item endorsed) or "high" ( $\geq 3$  PFC items endorsed) risk for depression-related difficulties were contacted. Once contacted, an additional phone screening assessing for the presence of neurological disorders (e.g., seizure disorder, closed head injury), autism spectrum disorders, developmental delays, premature birth ( $<36$  weeks gestation), and psychotropic medication use was completed. Endorsement of any of these conditions was exclusionary for all children. Using these screening criteria, 67 preschoolers were recruited into the study and 47 provided usable fMRI data. Of the 20 remaining children, fMRI data were lost because of failed QC ( $n = 18$ ), equipment failure ( $n = 1$ ), and discontinuation of scan per child request ( $n = 1$ ). Baseline data from these 47 children has been previously reported on in (Gaffrey et al., 2013). Of the 47 children with usable baseline fMRI data, 31 agreed to participate in an additional follow-up assessment approximately 1 year later, including 5 meeting for preschool depression and 26 healthy control children. On average, follow-up assessments took place  $364 (\pm 87)$  days following the baseline appointment. Of the 16 children not included, 8 preschoolers with depression were subsequently enrolled into a treatment study following their baseline scan which prevented their participation and 8 (5 healthy controls and 3 preschool depression) were unable to be contacted or refused to participate. Preschoolers with PO-MDD who did not participate in a follow-up assessment were not different from those who did in parent-reported negative affect ( $t[21] = -.493$ ,  $p = .627$ , 2-tailed) or amygdala reactivity to sad faces ( $t[21] = -1.5$ ,  $p = 0.15$ , 2-tailed) at baseline. Parental written consent and child verbal assent were obtained for all subjects. The Institutional Review Board at Washington University in St. Louis approved all experimental procedures.

### 2.2. Diagnostic assessment

Diagnostic assessments were conducted at baseline using the preschool age psychiatric assessment (PAPA), a developmentally appropriate, interviewer-based instrument designed for use with the primary caregivers of children between 2 and 7 years of age (Egger et al., 1999, 2003). The PAPA includes all relevant DSM-IV (APA, 2000) criteria and their age appropriate manifestations, has established test-retest reliability (Egger et al., 2006), and is widely used to assess for DSM-IV Axis I disorders in preschoolers. Detailed training and calibration methods have been previously described (Luby et al., 2009). After completion of the PAPA by trained research assistants, relevant symptom, impairment, and duration criteria gathered during the interview were used to generate diagnoses, including preschool depression (PO-MDD; Luby et al., 2014). Children placed into the control group did not meet criteria for any DSM-IV Axis I disorder according to parent report on the PAPA.

### 2.3. Measure of child's negative affect

Parents completed the emotion regulation checklist (ERC; Shields and Cicchetti, 1997) at baseline and follow-up. The ERC is a parent report measure of children's dysregulated negative affect (Negativity) and successful emotion regulation, and includes both positively and negatively weighted items to be rated on a 4-point Likert scale. Given our specific hypotheses about amygdala function and negative affect, the Negativity (Cronbach's  $\alpha = 0.84$ ) subscale was of particular interest and therefore was used in the analyses described below. The Negativity subscale is composed of 15 questions and possible scores range from 15 to 60 points. High scores reflect rapid changes in child mood (i.e., quickly moving from positive to negative moods), angry reactivity, and increased intensity of

emotion. Example items include, “Is easily frustrated” and “Is prone to angry outbursts/tantrums easily.”

#### 2.4. Maternal history of depression

The family interview for genetic studies (FIGS) was used to obtain maternal history of depression (Maxwell, 1992). The FIGS is a fully structured instrument for which the first author (M.S.G.) and senior investigator (J.L.L.) trained interviewers to reliability.

#### 2.5. Face viewing task

Approximately 7–10 days after their baseline visit, children participated in an fMRI scan where they were presented with a series of faces varying in affective content and asked to complete a simple button press each time a face appeared. Of the possible 43 unique individuals in the Nimstim Set of Facial Expressions (Nim-Stim; <http://www.macbrain.org/resources.htm>), 21 with available neutral, happy, sad, and fearful facial expressions were used and counterbalanced for gender and ethnicity. Children completed two 5.3 min scan runs of the face-viewing task. During each run 4 face blocks including 8 of the same face type (e.g., sad) were interleaved with 35-s fixation blocks, each face was presented for 3.5 s followed by a fixation cross for 1.5 s. See Gaffrey et al. (2013) for full details.

#### 2.6. Functional data acquisition and processing

Imaging data were collected using a 3T TIM TRIO Siemens whole-body system. To create familiarity and comfort with study procedures, each child was provided with a child-friendly video introducing the fMRI experience before their visit, introduced to the scanning environment using a mock scanner training protocol during their initial in-person assessment, allowed to watch a movie of their choice during structural scans, and rewarded with small prizes after scan completion. Image acquisition included an initial low-resolution 3D sagittal T1-weighted MP-RAGE rapidly warped to Talairach space (Talairach and Tournoux, 1988). This image was then used to provide online slice localization for the functional images, placing them as close as possible to the target template. T1 images were acquired as part of the structural imaging protocol, and were used in the transformation of images to a common template space optimized for preschool children. The accuracy and validity of this transformation for preschool age children has been demonstrated in previous research (Ghosh et al., 2010). The functional images were collected with a 12-channel head coil using an asymmetric spin-echo echo-planar sequence sensitive to blood oxygen level-dependent (BOLD) contrast ( $T_2^*$ ; repetition time [TR]=2500 ms, echo time [TE]=27 ms, field of view [FOV]=256 mm, flip angle=90°). During each functional run, sets of 36 contiguous axial images with isotropic voxels (4 mm<sup>3</sup>) were acquired parallel to the anterior–posterior commissure plane. Stimuli were presented using PsyScope X on an Intel Macintosh computer, with the start of each run directly triggered by a pulse from the scanner.

Before preprocessing, the first 4 frames of each run were discarded to allow for signal stabilization. The fMRI data were preprocessed and analyzed using in-house, Washington University software (FIDL analysis package, <http://www.nil.wustl.edu/~fidl/>). Data were reconstructed into images and normalized across runs by scaling whole-brain signal intensity to a fixed value and removing the linear slope on a voxel-by-voxel basis to counteract effects of drift (Bandettini et al., 1993). Data were also corrected for head motion using rigid-body rotation and translation correction algorithms (Friston et al., 1994; Snyder, 1996; Woods et al., 1992), co-registered to Talairach space using a 12-parameter linear (affine) transformation that included resampling to 3 mm cubic,

and smoothed using a 6 mm FWHM Gaussian filter. Within scan head movement was assessed using output from the rigid-body rotation and translation algorithm. After measuring the translations and rotations in the x, y, and z planes across frames, total root mean square (RMS) linear and angular measures were calculated and used to obtain the average amount of movement in millimeters from frame-to-frame (i.e., TR-to-TR) in a given run for each subject (RMS/frame). Face-processing runs with greater than 0.15 mm RMS/frame were excluded from further data analysis. This resulted in 6 children providing only 1 run of face processing data and a group average RMS/frame of 0.08 mm ( $\pm 0.02$  mm). To further reduce any potential effects of head movement on data quality, custom Matlab (Mathworks, Natwick, MA) code was used to identify frames with greater than 0.7-mm absolute movement (Siegel et al., 2014). The identified frames, as well as the frames before and after them, were removed from further data analysis resulting in an average loss of 10 ( $\pm 5$ ) frames per child.

#### 2.7. Data analysis

Estimates of functional activation during each condition were obtained using block-design analyses. This included the use of a general linear model (GLM) incorporating regressors for linear trend and baseline shift to estimate the hemodynamic response function for each stimulus type (i.e., facial expression). Within the GLM, a hemodynamic response shape was assumed (Boynton function) and used to derive percent signal change values for each stimulus type relative to baseline fixation, which were then used in all subsequent statistical analyses.

Following our primary interest in the relationships between amygdala activity and level of negative affect both concurrently and in the future, we chose to focus our analyses on the right amygdala given its identified relationship with parent reported negative affect in our previous report. Thus, based on our previous report (Gaffrey et al., 2013), a region-of-interest (ROI) was defined using a region of the right amygdala that had greater activity in depressed preschoolers when compared to their healthy peers and was also positively related to parent reported negative affect at the time of scan. Given that the group comparison contrast identifying this right amygdala ROI was orthogonal to the correlational analysis identifying its relationship with negative affect, it is not subject to the “double-dipping” confounds described by Kriegeskorte et al. (2009). In order to examine the specificity of any significant relationships found between the right amygdala and negative affect, a left amygdala ROI was also examined. In order to create the left amygdala ROI, the right amygdala ROI was ‘flipped’ by changing the original x-coordinate from positive to negative. Amygdala activity was measured as the percentage of change in the blood oxygen level-dependant (BOLD) signal (i.e., percent signal change) while viewing faces compared to baseline fixation.

Amygdala activity during face viewing was evaluated using a repeated measures analysis of variance (ANOVA) with face type (i.e., sad, happy, fear, neutral) as a within subject factor. Post hoc *t*-tests were planned if a significant main effect of face emotion type was found to identify which face types differed. Given the unique age of the current sample, and the limited data available to inform amygdala activity during face processing at this age, exploratory *t*-tests were also planned to assess for differences between face emotion types if a significant main effect of face emotion type was not present.

The relationship between individual differences in baseline negative affect, as measured by the ERC Negativity subscale, and functional activity of the amygdala while viewing individual face emotion types were evaluated using the Pearson product-moment correlation coefficient (*r*). Given our previous work (from which the current sample was drawn) suggesting that greater amygdala

activity is related to increased negative affect at the time of scan (Gaffrey et al., 2013), a 1-tailed approach was used to determine significant correlations between variables. To correct for multiple comparisons, an adjusted  $p$ -value was generated based on the 4 face emotion types for each amygdala ROI (adjusted  $p < .0125$ ). Hierarchical linear regression analyses were used to examine the relationship between amygdala activity at baseline and negative affect at 12-month follow-up. Only associations between amygdala activity and negative affect at baseline surviving correction were explored as independent variables in the regression analyses. Regressions included negative affect at 12-month follow-up as the dependant variable. Negative affect at baseline was entered in the first step followed by amygdala activity in a subsequent step.

### 3. Results

#### 3.1. Demographic, negative affect, and diagnostic characteristics

Children were split evenly by gender ( $n = 16$  female), age 5.5 ( $\pm 0.9$ ) years old on average at their baseline assessment, and ethnicity was primarily Caucasian ( $n = 26$  Caucasian, 2 African American, 3 multiracial). Five children met criteria for PO-MDD based on the PAPA. In addition to meeting for PO-MDD, 2 children also met for Generalized Anxiety Disorder and 1 child also met for Conduct Disorder, ODD, and Separation Anxiety Disorder according to the PAPA. Average ERC Negative Affect scores were 27.3 ( $\pm 6.3$ ) at baseline and 23.5 ( $\pm 5.3$ ) at follow-up, with an average drop of 3.7 ( $\pm 6$ ) points from baseline to follow-up (calculated as baseline minus follow-up). Thus, as a group children showed a decline in negative affect over the course of follow-up.

#### 3.2. Amygdala activity while viewing facial expressions of emotion

The repeated measures ANOVA did not reveal a significant main effect of face emotion type for the left ( $F_{(3,90)} = 0.273$ ,  $p = 0.85$ ) amygdala, though there was a trend for the right amygdala ( $F_{(3,90)} = 2.27$ ,  $p = 0.085$ ). Exploratory follow-up  $t$ -tests indicated that right amygdala activity while viewing sad faces was greater than that for happy faces ( $t[30] = 2.043$ ,  $p = 0.05$ , 2-tailed; see Fig. 1).

#### 3.3. Amygdala activity and negative affect

A multivariate approach to identifying potential outliers using Mahalanobis  $D^2$  was conducted prior to carrying out a priori correlational analyses including amygdala activity for each face emotion type and ERC Negativity subscale scores at baseline. No outliers were identified. When significant correlations between baseline amygdala and negative affect scores passing our corrected  $p$ -value were identified, the relationships between amygdala activity for the identified face types and ERC Negativity scores at follow-up were examined for outliers prior to use in regression analyses. This revealed 1 outlier when sad face right amygdala activity and Negativity at follow-up were examined. This outlier was then excluded from the subsequent regression analysis including right amygdala sad face activity.

#### 3.4. Amygdala activity and negative affect at baseline

Correlational analyses revealed a positive relationship between baseline Negativity scores and right amygdala activity (see Fig. 2) during sad face viewing ( $r[31] = 0.45$ ,  $p = .005$ , 1-tailed), right amygdala activity during happy face viewing ( $r[31] = 0.43$ ,  $p = .008$ , 1-tailed), and left amygdala activity during sad face viewing ( $r[31] = 0.53$ ,  $p = .001$ , 1-tailed).

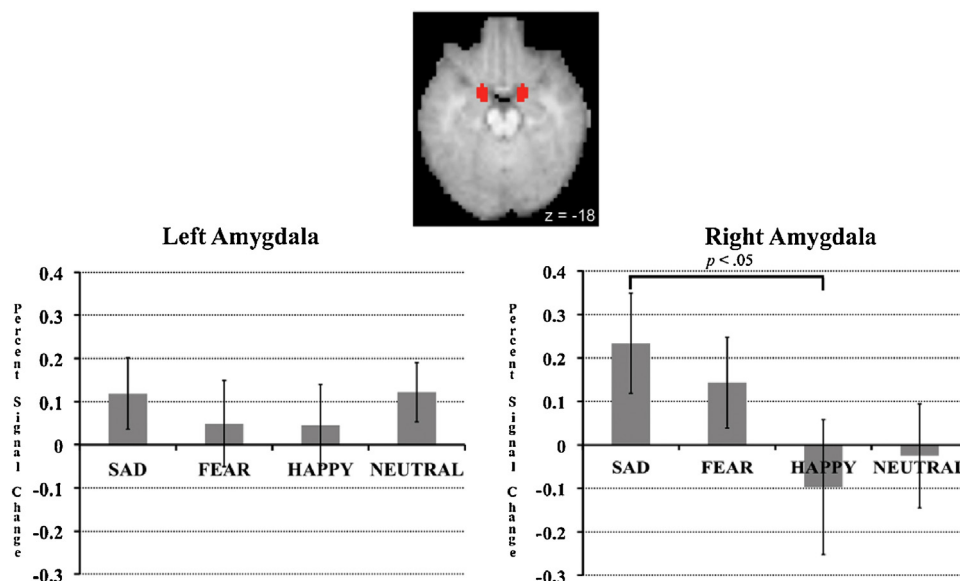
#### 3.5. Amygdala activity and negative affect at 12-month follow-up

Hierarchical regression analyses indicated that right amygdala activity during sad face viewing predicted parent reported Negativity scores at follow-up, even after controlling for parent reported negative affect reported at baseline (see Table 1 and partial regression plot of residuals in Fig. 3). In addition, right amygdala activity

**Table 1**

Right amygdala activity to sad faces predicts negative affect 12-months later.

Regression step	$R^2_{\text{adjusted}}$	$B$	SE	$\beta$	$p$
Negative affect at follow-up					
Step 1	.277				.002
Baseline Negative Affect		.479	.138	.550	.002
Step 2	.431				.007
Baseline Negative Affect		.338	.131	.388	.016
Right amygdala sad face		4.532	1.55	.441	.007



**Fig. 1.** Amygdala activity to specific facial expressions of emotion.



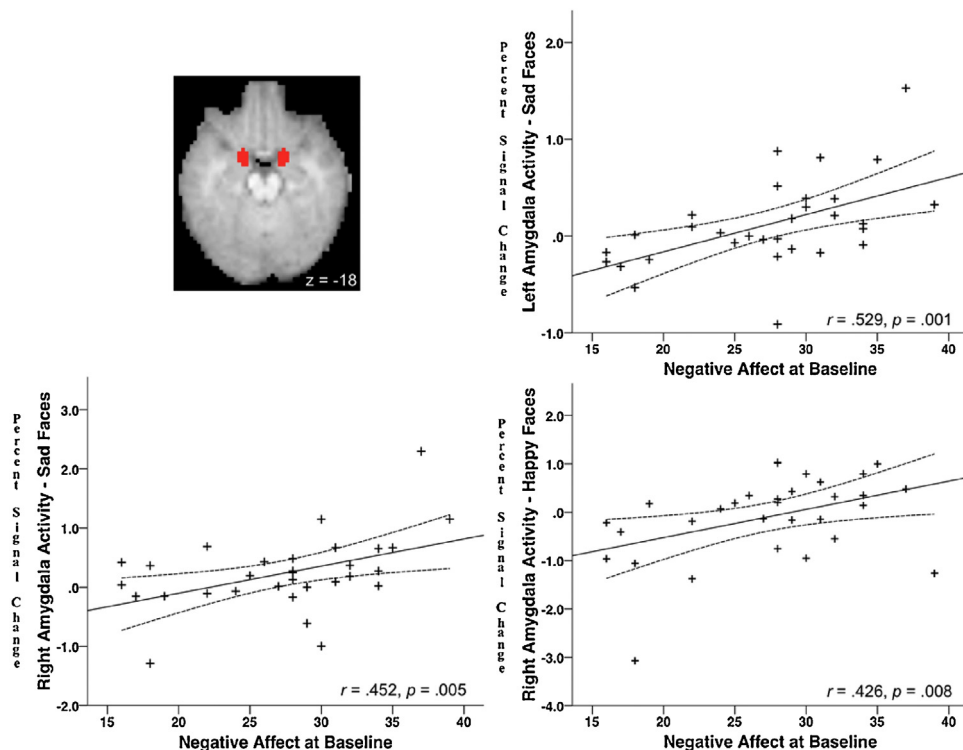


Fig. 2. Amygdala activity is related to baseline parent-reported negative affect in 4–6 year old children.

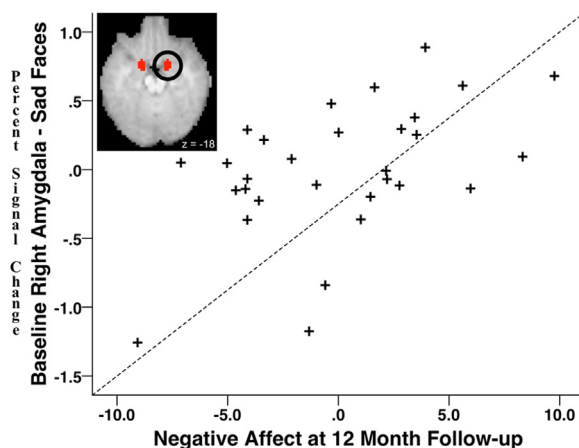


Fig. 3. Partial regression plot illustrating that right amygdala reactivity to sad faces continues to predict parent-reported negative affect at 12-month follow-up after baseline parent-reported negative affect is controlled.

to sad faces remained a significant predictor of follow-up Negativity when baseline diagnostic group status (i.e., no diagnosis vs. PO-MDD;  $R^2_{\text{adjusted}} = .435, p = .013$ ) or maternal history of depression ( $R^2_{\text{adjusted}} = .413, p = .007$ ) were also entered into the first step of the hierarchical regression with baseline Negativity scores and when the 5 preschoolers with PO-MDD were excluded ( $R^2_{\text{adjusted}} = .250, p = .023$ ). Right amygdala activity during happy face viewing ( $R^2_{\text{adjusted}} = -.010, p = .412$ ) and left amygdala activity during sad face viewing ( $R^2_{\text{adjusted}} = .008, p = .274$ ) did not significantly predict Negativity scores at follow-up.

#### 4. Discussion

The goal of the current study was to examine the relationship between amygdala activity during face viewing and the

early developmental course of negative affect in preschool age children. Dimensional analyses revealed significant relationships between right amygdala activity while viewing sad faces and parent-reported negative affect both at the time of scan as well as 12-months later. Importantly, right amygdala activity to sad faces predicted negative affect 12-months later even after controlling for parent reported level of negative affect and diagnostic status at baseline. In other words, heightened right amygdala activity to sad faces exhibited robust predictive power for elevated negative affect measured approximately 1 year later even when dimensional and categorical measures of parent-reported negative affect were accounted for. To our knowledge, this is the first study to demonstrate a predictive relationship between amygdala function and later negative affect in preschool age children.

Research using latent growth curve modeling has suggested that the developmental trajectory of negative affect follows a predictable structure throughout childhood, with negative affect peaking during early childhood and then steadily declining through the school age period (Olino et al., 2011; Partridge and Lerner, 2007). These models have also suggested significant individual variance in this trajectory both cross-sectionally and longitudinally. More specifically, where a child falls relative to their peers is likely to remain consistent across childhood (Neppi et al., 2010). For example, a toddler with relatively higher negative affect than their peers is likely to develop into a preschooler with the same profile. Interestingly, recent findings also suggest that a small percentage of very young children may also go on to exhibit further increases in negative affect as they age, suggesting not only persistently elevated negative affect compared to peers but also a worsening of functioning over time (Wiggins et al., 2014). The current study extends these findings by suggesting that early amygdala reactivity to sad faces may be a promising biomarker and predictor of individual changes in the developmental trajectory of negative affect. Importantly, the current study provides the first link between amygdala function and developmental change in negative affect in very young children. While future longitudinal research

including measures of both amygdala function and negative affect at multiple time points will be needed to fully examine how the developmental trajectories of each are related, the current study suggests that measuring amygdala activity during face viewing is likely to be a highly fruitful approach for capturing an early neural marker of developmental changes in negative affect during early childhood.

Recent research has suggested that elevated irritability and negative emotionality early in life are significant predictors of later depression, including depression occurring during school-age, adolescence, and adulthood (Dougherty et al., 2013; Bould et al., 2014; Karevold et al., 2009). This work has also suggested that those at greatest risk for depression are likely to demonstrate persistently high or increasing levels of negative affect over the course of early childhood (Wiggins et al., 2014), a period when negative affect is typically decreasing. The current findings suggest that variations in amygdala activity may be one of the mechanisms contributing to such alterations in negative affect, and thus has the potential to significantly inform the early identification of risk for later psychiatric illness. The search for early neural markers of later psychopathology has been prioritized based on the potential to intervene more effectively during periods of relatively great brain plasticity (Insel, 2014). While the current study does not directly inform whether amygdala activity can serve as a predictor of future *diagnostic* status, it does raise the intriguing possibility that functional activity within the amygdala may serve as a significant biomarker that can identify children with increased- or increasing-risk for later negative affect and potential for related psychiatric disorders. However, while the current study represents an important step forward, many pragmatic as well as scientific questions will need to be answered before the potential of amygdala activity, an expensive and somewhat difficult measure to obtain, will be useful to inform earlier identification of emerging psychiatric illness.

Nevertheless, while the use of neuroimaging data to directly inform psychiatric risk within a clinical setting is still an open question (Bullmore, 2012), the current findings highlight the potential of fMRI data to further clarify whether and to what extent the developmental trajectories of negative affect and amygdala function are related. Such information may prove to be important for developing clinical procedures that identify individual children exhibiting increasing risk for (or the early emergence of) psychiatric difficulties. More specifically, as our understanding of the specific relationship between negative affect and amygdala development grows, leveraging this knowledge to identify other neuropsychological and/or physiological measures that can be used effectively and potentially more affordably within clinic settings to identify emerging psychopathology and/or response to treatment may be increasingly possible (Casey et al., 2014). In line with an experimental therapeutics approach to treatment development (Insel and Gogtay, 2014), identifying specific brain-behavior relationships in health as well as disorder is considered foundational to such an effort. The current study provides one of the first pieces of evidence supporting a specific relationship between amygdala function and current as well as future negative affect in preschool age children. Future work examining this relationship and factors influencing it is now needed.

Some limitations should be mentioned. It will be important for future efforts to include larger samples of children and multiple time points including measures of negative affect, positive affect, and amygdala activity. Doing so would allow for the needed replication of the current findings and allow for a more thorough and specific examination of the interaction between negative as well as positive affect and amygdala reactivity over the course of early development. This type of approach may also help to further clarify the relationship between increased amygdala reactivity to happy faces and concurrent negative affect found in the current study.

While amygdala reactivity to happy faces did not predict future increases in negative affect, its relationship with baseline negative affect raises the intriguing possibility that heightened amygdala reactivity to emotionally relevant stimuli that are highly salient and evocative during this developmental period (e.g., sad and happy faces) may reflect state as well as trait markers of negative affect. Likewise, a study of this nature would also enable the potential prediction of later psychopathology as children age, something not possible with the size of the current sample and the relatively short period of follow-up. Given that the amygdala is one node within an extended corticolimbic network important for emotion and emotion regulation, the inclusion of measures of amygdala connectivity would also likely benefit future research. Nevertheless, the current study represents an important step forward in our developmental understanding of early childhood negative affect and its underlying neurobiology.

## 5. Conclusion

In summary, the current study provides the new evidence demonstrating that amygdala function in preschool age children predicts parent reported negative affect concurrently and 12 months later. Importantly, these predictions remained significant even after controlling for key baseline behavioral measures. These findings are generally consistent with previous reports indicating an important role for the amygdala in early emotional development and provide preliminary evidence for amygdala activity as a potential biomarker of persistent negative affect. The need for early and objective markers of risk for later internalizing psychopathology is clear. Whether neural markers prove more robust or feasible to serve as clinically relevant markers in early childhood is an important area for future investigation.

## Conflict of interests

The authors have no financial interest(s)/conflicts to disclose. M.S.G. had access to all study data and takes responsibility for data analysis integrity/accuracy.

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